From: <u>Bill Jacobs</u>
To: <u>Alex Wegmann</u>

Cc: <u>Aurora Alifano</u>; <u>Gregg Howald</u>

Subject: Re: more bait trials at palmyra - would appreciate your 2 cents on study design

Date: 02/12/2010 05:25 PM
Attachments: Palmyra Bait Studies 2010.pdf

I have held off replying to this note because I had hoped to be able to attach some protocols to it. However, we were snowed out all week (until today -- which happened to be a day off for me but I'm working at home anyway). At this point, I'll provide some comments on the attachment to Alex's note of 2/4/10. I'll provide some efficacy protocols later and see what I can do about the soil residue part, which is handled by our environmental chemists.

Our efficacy protocols are designed to pit candidate bait formulations against a standard challenge diet, the contents of which could only be approximated on Palmyra by rats who had access to the kitchen or other stores of human foods. Essentially, that means a subset of Cooper's rats.

Clearly, what is important on Palmyra is that the bait used be attractive enough to all rats that they will consume fatal dosages. From the 2005 and 2008 trials, it seems clear enough that 25W baits and/or placebo baits for it were taken well by rats on Home, Portsmouth, Whippoorwill, Bunker, etc., with availability rather than palatability being the likely reason for incomplete saturation of collected rats with marker in the 2008 trials on Whippoorwill and Home. The Diphacinone bait might or might not correspond to the formulation used in the 2004 trials.

The choice of alternative diets that Alex proposes seems reasonable to me. Maintaining them on peanut butter alone might be an issue. Peanut butter on something might be better, or laboratory diet (which the rats on Wake -- unlike others in my experience -- really went for). (Araceli and I worried that peanut butter on ancient uncooked Stove Top Stuffing mixture that we used in 2008 might not be life-sustaining, but none of our rats died in captivity prior to sacrifice; and the juvenile that we had on it gained 19 g in 7-8 days.)

The proposed method of stimulus presentation seems OK in a situation in which assessments of preference are to be mostly qualitative. If the diets were not accessible from wires with equivalent ease, a source of bias could be introduced.

The transparent caging should facilitate observations but might create problems if there were no way to transfer rats in and out of them without risking escape. It was a lot easier transferring Palmyra's rats from Haguramas (sp?) to Tomahawks than it was transferring them to the various cage designs that were created on the spot.

Presumably, the plan is to assess dietary preferences for rats collected on Cooper. It might be useful do at least a mini-replicate of the experiment on another island where there is cover (e.g., on Bunker or Whippoorwill), if feasible.

The plastic cages or the old Talon buckets would work for the hermit crab trials which, I suggest, should include the purple species as well as the reddish one. (So should the excrement studies. Presumably, there is some sort of ecological division of labor between the two species which allows them to coexist. Whatever that division might be is not readily apparent.)

In addition to crab excrement and soil, there are other things which likely would be contaminated through bait applications and might prove to be important routes of transfer of active ingredient to nontarget species. In the biomarker work, we found evidence of marker in every type of potential primary consumer that we examined. We found no biomarker in geckos, but we also found no evidence in the many rats that we cut up of marker anywhere that it would have to have gotten systemically. We didn't see fluorescence in rat livers or other internal organs that are not part of the GI tract, even in the rats that we sacrificed after 2-4 days on bait *ad libitum*. Yet we know that anticoagulants go to liver and muscle. Whatever happened metabolically to pyranine once it was ingested did not appear to result in systemic transfer of fluorescent material. Therefore, that the geckos looked clean in the 2008 trials does not mean that they would be unaffected by rodenticide applications. Clearly, the same would go for birds that eat crabs, ants, katydids, geckos, and poop from the foregoing as well as other primary and secondary consumers.

While neither of the compounds is safe for nontarget vertebrates, Diphacinone is far more forgiving to birds than Brodifacoum is. It also is somewhat more forgiving to rats, but probably only significantly so to rats that have the resistance factor. As part of the 2010 investigations, it would be a good idea to collect DNA samples from all rats used in trials (and others trapped for the purpose) to have them examined for presence of alleles that confer resistance to anticoagulants. Prior anticoagulant use on Palmyra can only select for resistant animals if a resistance factor is present, which seems fairly likely on its face because WW2 activities (for example) could have resulted in multiple introductions of roof rats. Still, the matter should be researched because the presence or absence of resistance factor is a critical bit of information to have when choosing between a first- and a second-generation anticoagulant.

Feeding the anticoagulant baits to a few crabs should be done to verify that the baits do not kill them. Although there are reasons to believe that anticoagulants do not affect invertebrate due to their open circulatory systems, it might be that the rodenticides or something else in one or both of the baits kill crabs in some other way. Poisoned crabs would become separated from their shells and, therefore, more accessible to some predators and scavengers.

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Alex to: Bill Jacobs Wegmann

02/04/2010 05:46 PM

Cc: "Gregg Howald", "Aurora Alifano"

To inform several aspects of the Palmyra rat removal EIS, we (FWS, TNC, and IC) have decided to conduct more bait trails at Palmyra. Our on-island window is 9 May to 10 June 2009. During this time, we intend to do the following:

- 1. Measure the palatability (for rats) of both registered conservation bait products (Brodifacoum 25-W and Diphacinone-50) against naturally occurring food items (coconut, Pandanus fruit, Terminalia fruit)
- 2. Measure the palatability (for crabs) of Brodifacoum 25-W and Diphacinone-50) against naturally occurring food items (coconut, Pandanus fruit, Terminalia fruit)
- 3. Measure the palatability (for rats) of excrement from crabs post feeding on Brodifacoum 25-W and Diphacinone-50
- 4. Measure toxicant residue in two soil types over time: sandy, humus
- 5. Measure toxicant residue in crab excrement over time

I have attached a PDF file that outlines the studies. This is as far as we've gotten with planning the studies, which is why I'm knocking on your door for advice. Bill, do you have any of the following that you can send to me:

- EPA or other industry standards on rodent palatability trials
 - o I realize that we won't be able to exactly replicate lab-based standards while conducting the trials at Palmyra (you know what the conditions are like down there), but we want to be as close to "standard" as possible.
- EPA or other industry standards on sampling toxicant residue in soil

And, it goes without saying, that your general (or specific) thoughts on these studies are more than welcome.